

ART. XI.—*Studies in the Physiology of Host-Parasite Relations.*

II. ADVENTITIOUS ROOT FORMATION.

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Introduction.

Recent advances in the study of the response of healthy plants to growth substances have suggested the possibility of their being concerned in similar stimulation effects induced in plants infected by certain bacterial pathogens.

Three pathogenic organisms which induce atypical growth response in some host plants are *Bacterium solanacearum* (Bacterial Wilt of Solanaceae), *Aplanobacter michiganense* (Bacterial Canker of Tomato) and *Bacterium tumefaciens* (Crown Gall).

The production of adventitious roots on intact stems of tomato is induced by all three organisms, epinasty of leaves and stimulated cambial activity are induced by *B. solanacearum* and *B. tumefaciens*, while cell proliferation leading to gall formation is characteristic of *B. tumefaciens*.

In an earlier paper (Grieve, 1939) the epinastic response of leaves, induced by *B. solanacearum*, was examined and the question of its proximal cause discussed. Epinastic response of leaves, adventitious root formation and cambial stimulation induced in plants by *B. tumefaciens* and the relations of these effects to growth substance has been studied by Locke, Riker, and Duggar (1938). The present paper deals firstly with observations on the development of adventitious roots in plants invaded by *Bacterium solanacearum* and secondly with their relation to growth substance, comparisons being made with similar effects induced by humidity, gravity, wounding, blocking, synthetic growth substances and by the two other organisms referred to above.

That tomato plants react to invasion by *B. solanacearum* by the formation of adventitious roots was first indicated by Hunger in 1901. His observations were confirmed by Smith (1914, 1920) who demonstrated that adventitious roots arose above and below the point of inoculation whereas no adventitious roots developed on control stems pricked with a sterile needle. Smith also

demonstrated that adventitious roots developed on infected tobacco stems. Bryan (1915) while working with tall and dwarf varieties of garden nasturtium (*Tropaeolum majus*) observed that the tall varieties reacted to bacterial invasion by adventitious root formation.

Hutchinson (1913) and Smith (1920) made tentative suggestions as to the proximal cause of this stimulation effect but no critical investigation appears to have been attempted.

The present work was commenced when the characteristic development of the roots was observed in artificially infected tomato plants (Grieve, 1936A).

Methods.

In the case of *Bacterium solanaccarum* the technique of inoculation, the cultures used and the methods of fixing and staining infected material were as described in an earlier paper (Grieve, 1939). The culture of *Aplanobacter michiganense* used was a strain isolated from tomato in Victoria by A. Pugsley of the Department of Agriculture, and the Crown Gall organism was a strain isolated from almond (Grieve, 1934).

Test plants were grown in a glasshouse during spring and summer months, minimum night temperatures being approximately 58°F. and average day temperatures ranging from 65°F. to 80°F. The following plants were examined for adventitious root formation after infection by *Bacterium solanaccarum*:—*Lycopersicon esculentum* Mill., *Solanum tuberosum* L., *Solanum nigrum* L., *Solanum dulcamara* L., *Tagetes erecta* L., *Helianthus annuus* L., *Tropaeolum majus* L., *Ricinus communis* L., and *Impatiens balsamina* L.

After determining experimentally which plants showed the reaction, further experiments were made with those in which it developed most conspicuously. Tomato plants only were used for observing stimulation effects due to *Bacterium tumefaciens* and *Aplanobacter michiganense*.

For observations on the outgrowth of adventitious roots under humid conditions the plants were placed under bell jars or in large glass cases.

To ascertain the effect of gravity on adventitious root formation in tomato, experiments were performed in which some plants were staked horizontal and others rotated on a klinostat in order to neutralize the effect of gravity. All these plants were kept under humid conditions.

Experiments to determine the concentration of β -indole-acetic acid necessary to cause approximately equivalent adventitious root formation on tomato and other plants were made using water

solutions contained in small glass tubes drawn to a capillary at one end. The capillary end of the tube was inserted into the stem in the vicinity of a vascular bundle and its contents allowed to drain slowly into the plant. Each tube had a capacity of about 0.4 cc. and the concentration was 0.03 per cent. Lanoline-water emulsions of β -indole-acetic acid were used in some of the experiments relating to the effect of gravity and of growth substance on adventitious root formation.

The presence of growth substances in liquid media in which cultures of the organisms named earlier had been grown (for composition of media used see Grieve, 1939) was detected using the ether extraction procedure of Thimann and Bonner (1933) with *Avena* coleoptile tests. The *Avena* tests were made according to Went's technique (1928) and "Victory" oats were used. The husked oats were soaked for two hours, then the water was poured off and they were left in light for a further seven hours. They were then transferred to petri dishes lined with moist filter paper and germination allowed to proceed for about 30 hours. By this time the coleoptiles and roots were well developed and the seedlings were planted in individual holders with their roots in water and grown in a compartment held at 25°C. and 90 per cent, relative humidity. When the coleoptiles had reached a length of approximately 3 cm. they were ready for use and the standard method of decapitation and application of the agar blocks mixed with extracted growth substance was followed. Dolk and Thimann's method (1932) of preparation of agar blocks mixed with extracted growth substance was used, the final volume of each block for application to a decapitated coleoptile being 10.7 cmm.

For comparing growth substance content of stem parts of inoculated and healthy tomato plants the diffusion method was first tried, in which comparable stem parts from test and control plants were placed on agar plates of a size $8 \times 10.7 \times 1.5$ mm. for two hours. Each agar plate was then cut into twelve small blocks of equal size and these applied to decapitated coleoptiles. Satisfactory results for tomato were only obtained, however, when stem portions close to the apex were used. Overbeek's ether extraction method (1938) was next tried and as more satisfactory results were obtained it was used for most of the experiments. In comparing the growth substance content of the plants, equal fresh weights of stem parts were extracted separately.

The experiments on mechanical blocking of bundles were made using cocoa butter, lanoline and a paraffin wax-vaseline mixture. The blocking substances were introduced in the melted state through incisions in the plant stems.

Observations on Adventitious Roots in Healthy Plants.

Adventitious roots may be defined as those which arise in unaccustomed places and for the purposes of this paper are limited to those which develop on the stems of intact plants. In origin they are endogenous, developing by the formation of apical root meristems from the pericyclic regions in the vicinity of the outer phloem groups. Owing to the fact that these adventitious roots sometimes develop on healthy plants, a study was first made of some of the determining factors. Observations extending over fortnightly periods, were made on batches of tomato, sunflower, garden nasturtium, balsam, black nightshade, African marigold and potato plants of varying ages which were growing quite straight in the glasshouse. Only a very sparse development of adventive root primordia was observed in tomato, African marigold and balsam plants and none in the other plants.

The adventitious roots visible at the surface as small nodular projections covered by the epidermis, were confined to the basal internodes in tomato and to 1 to 2 inches above soil level in African marigold. The plants were next grown for fourteen days in highly humid conditions under bell jars and glass cases. Only a slight increase in the number of root primordia was observed. Very rarely did these root anlage pierce the epidermis and grow out into the humid atmosphere. It has frequently been observed in tomato plants which bend over sideways (often as a result of watering) that adventitious root primordia develop in some abundance along the lower side of the stem. This suggested that gravity was an important factor and experiments extending over fourteen days were performed in which stems of tomato plants were staked horizontally, both under normal and under very humid conditions. Root primordia developed on the lower side of the stems over several internodes up to the point where the unstaked tips turned upwards. This observation confirmed that made earlier by Laibach and Fischnich (1935), but the experiment was next carried further by rotating tomato plants with their stems held in a horizontal position on a klinostat for ten to fourteen days. No trace of adventitious root formation, even under very humid conditions occurred over the length of such horizontally rotated stems. These experiments made it clear that gravity and not humidity was the dominant causal factor in adventitious root formation in tomato. The development of these roots on the lower side of the horizontally staked stems where gravity is exerting its effect, was suggested by Laibach and Fischnich (1935) to be due to some movement of root forming substance from the upper to the lower side. In the experiments where the plants were rotated on the klinostat, each side of the stem was successively subjected to the influence of gravity and it is suggested that under these conditions no such localized accumulation of root forming substance can occur and no adventitious roots develop. Support for this view was given by

further experiments in which β -indole-acetic acid in lanoline was smeared for 1 inch along the upper surfaces of plant stems staked horizontally and for a comparable distance along one side of stems of plants ready for rotation on the klinostat. In the former case, root primordia developed over the upper treated region as well as along the lower surface, while in the rotated stems root primordia developed only along the line of growth substance smear. It was observed, even in the plants staked horizontally under humid conditions and where numerous root primordia became visible at the surface, that these only occasionally broke through the epidermis and grew out into the moist air. When such stems were staked horizontally in contact with moist soil or moss, however, the adventitious roots grew out speedily. These results suggest that contact stimulus is important in relation to the outgrowth of the roots which develop in response to gravity effect. It should be noted that this contact stimulus is not necessary for the outgrowth into humid air of adventitious roots when these are induced by growth substances or by bacterial infection.

Having defined the conditions and degrees of development of naturally forming adventitious roots in test plants, attention was next directed to ascertaining those host plants in which bacterial infection by *Bacterium solanacearum* stimulated definite root formation.

Range of Host Plants which Develop Adventitious Roots.

The pathogen *B. solanacearum* was inoculated either at the apex or base of the stems of test plants. Control plants were pricked in the same regions with a sterile needle to serve as a check on normal adventitious root formation. Conditions in the glasshouse were such that the air in the vicinity of the plants was humid but no attempt was made to place the plants under very humid conditions. Results are expressed in Table I.

TABLE I.—TEST FOR ADVENTITIOUS ROOT FORMATION IN PLANTS INFECTED BY *B. solanacearum*.

TEST PLANT.	RESULT.
<i>Lycopersicon esculentum</i>	+
var. Marglobe	+ + +
.. Burwood Wonder	+ +
.. Dwarf Champion	+
<i>Trapaecolum majus</i>	
Tall variety	+ +
Dwarf variety	+
<i>Typhes erecta</i>	+ +
<i>Helianthus annuus</i>	+
<i>Solanum nigrum</i>	
.. <i>dubautiana</i>	
.. <i>tuberosum</i>	
<i>Ricinus communis</i>	
<i>Impatiens balsamina</i>	

+ + + signifies a large number of induced roots. signifies no roots.

The tomato variety Marglobe consistently showed the greatest number of adventitious roots developing, both above and below the inoculation point, and was consequently used as the main test variety for the experiments to be described later. The observations of Bryan (1915) regarding adventitious root formation on infected tall varieties of *Tropaeolum majus* were confirmed and it was also demonstrated that under the conditions of slow invasion in some of these experiments adventitious root formation could occur in dwarf varieties. The records of root formation on *Tagetes erecta* and *Helianthus annuus* are new. The effect was so definite in the former case that this plant was selected for additional experiments. Considerable difficulty was experienced in obtaining successful inoculations on sunflower plants, but in those cases where the disease occurred root primordia were found developing along the invaded bundles. Control plants pricked with a sterile needle showed a complete absence of such root primordia. No confirmation was obtained for the report by Stanford and Wolf (1917) that *Impatiens balsamina* reacted to invasion by adventitious root formation. The inoculation points were $1\frac{1}{2}$ to 2 inches above soil level and no roots developed above this level. The occasional development of roots, at or about soil level, was found to occur both in infected and healthy plants when held under humid conditions. Infected balsam plants showed marked browning of vessels (visible externally) due to gum formation, this being followed by wilting of leaves. Plants of *Solanum nigrum* and *Solanum dulcamara* wilted without development of roots, even when placed under conditions favouring slow invasion. Infected potato plants showed the stimulation response of leaf epinasty but no trace of adventitious root formation on the stem was observed.

Considerable variation, even between plants of closely similar size and vigour, was found in the period for the development of bacterially induced roots to the stage where they were clearly recognizable at the surface. This variation is probably due to the more rapid growth of the pathogen in one plant than another owing to more favourable implanting.

For tomato the minimum time for the adventitious roots to show as nodular swellings under the epidermis was found to be four days. This was recorded from three Marglobe tomato plants growing under conditions suitable for fairly rapid invasion. The majority of records, however, showed that six to ten days elapsed after inoculation before the roots were clearly distinguishable. Longer periods, extending from 14 up to 28 days, were recorded under conditions of slow invasion during autumn months. Under environmental conditions which favoured rapid invasion wilting occurred in tomato plants (6 to 8 inches tall) without any sign of root formation. Sections of stems of such plants failed to show any indication of the start of root primordia. Younger

tomato plants (3 to 4 inches tall) wilted without developing adventive roots even when invasion was retarded. Under optimum conditions invasion progressed more slowly in African marigold than in tomato, the minimum time recorded for root development to the nodule stage was fifteen days. The average time was 26 to 30 days. The invading bacteria were seldom present in the stem vessels in "blocking" numbers as in tomato and their rate of movement in the stem was much slower. The adventitious roots which developed were quite comparable in numbers to those on tomato plants.

REGIONS OF DEVELOPMENT OF ADVENTITIOUS ROOTS.

In naturally infected tomato plants adventitious roots developed characteristically along the path of the primary bundles, spreading later to the secondary tissues. Under conditions of moderately rapid invasion in plants 8 to 10 inches tall (wilting commencing in 12 to 15 days), adventitious roots were observed over as many as seven or eight internodal regions. They did not commonly develop in apical regions. Artificial inoculations by needle prick were generally made in the vicinity of the primary bundles. Definite root formation occurred both above and below the prick as the bacteria multiplied and the bacterial columns spread upwards and downwards in the vessels. Hunger (1901) observed that adventitious roots developed on tomato leaf petioles as well as on the stem. In the course of this investigation, however, none has been seen to develop on leaf petioles.

The root primordia which developed were very variable in number on different tomato plants. The average number was between 40 and 50, extending over four to five internodes, with maximum numbers of roots ranging up to 200. On the other hand as few as five to six primordia definitely associated with the disease were recorded. In control plants as many as five to six naturally occurring roots were recorded in basal internodal regions.

For African marigold and garden nasturtium the development of the induced roots was along the course of the bundles and the numbers developing were comparable in any internode to those on tomato. In sunflower the numbers of induced roots were few.

THE EFFECT OF HUMIDITY ON THE OUTGROWTH OF BACTERIALLY INDUCED ROOTS.

Under normal glasshouse conditions the individual roots develop no further than nodular projections covered by the epidermis, but when test plants are placed under humid conditions in glass cases or under large bell jars a large proportion, but not all, of the nodules force their way through the epidermis and grow out into the humid atmosphere. The length to which these roots grow out varies from $\frac{1}{4}$ inch to 2 inches. It was noticeable that

the greatest tendency for strong outgrowth of roots was in the lower internodes, but outgrowth from apical internodes was also recorded (Pl. XVI., fig. 1). Examination of Pl. XVI., figs. 1, 2 and 4, show that both in tomato and African marigold a number of the nodules fail to grow out. Sections through these have shown the presence of bacteria in large numbers in the vessels behind the root primordium. After some days the tips of several of the roots which grew out showed browning and no further growth occurred. Quite a long period elapsed however before any further breakdown changes were observed, even though test plants were in many cases badly infected and showing bacterial ooze at the surface of the stem.

In other experiments stems of infected plants were cut near the base and placed in a beaker of water under a bell jar. A much higher proportion of the nodules broke through the epidermis and grew out into the humid air (Pl. XVI., figs. 1 and 4). This increased outgrowth as compared to that on intact plants may be due, at least in part, to the diffusion of the "blocking" bacteria from the vessels allowing the transport of more water to the root primordia. As indicated earlier, intact healthy plants held under humid conditions have only rarely shown outgrowth of adventitious roots and these also were confined to the basal internodes. When cut stems of such plants were placed in water, roots developed in considerable numbers below the water surface. This contrasted strikingly with the appearance of infected plants, where the adventitious roots developed conspicuously on the stem both above and below the surface of the water.

Comparison of Adventitious Root Formation by *B. solanacearum*, *B. tumefaciens* and *A. michiganense*.

Adventitious root formation in tomato plants inoculated with *B. tumefaciens* did not compare in numbers, or in longitudinal distribution on the stem, with those induced in similar plants infected with *B. solanacearum*. Even when inoculations were made at five or six points on the stem as Locke, Riker and Duggar (1938) recommended, the numbers of roots, which were closely associated with the galls, did not approach in numbers those induced by the bacterial wilt organism. This difference in numbers and distribution is of course related to the rather localized nature of invasion in Crown Gall, as against the systematic development in bacterial wilt of Solanaceae. When placed in a humid atmosphere, outgrowth of the root nodules occurs in the same manner as do those in plants infected with *B. solanacearum* but no browning and breakdown of the outgrown roots was seen to occur in Crown Gall. No evidence has so far been presented to show that *B. tumefaciens* is present at the base of such developing roots as is often the case for *B. solanacearum*.

Adventitious root development induced in tomato by *Aplanobacter michiganense* was variable. In some cases the numbers were comparable to those induced by the bacterial organism causing wilt, but more often the numbers visible at the surface were few, and swelling and cankering of the stem occurred. Under humid conditions comparatively few of the root nodules grew out into the moist air and most of those that did so, rapidly browned and withered. Longitudinal and transverse sections through such roots showed the parasite invading and corroding the vascular system of the root. *A. michiganense* is primarily a phloem parasite and consequently is in a favourable position to attack root primordia shortly after they commence development. Even at the nodule stage, considerable corrosion of the vascular tissues of the embryo root has been found to occur, this being responsible for their failure to develop further when placed under humid conditions.

Comparison of Bacterially Induced Roots and those induced by Growth Substance.

During the course of the above experiments comparative tests were made on selected plants using a synthetic growth substance (β -indole-acetic acid) applied in aqueous solution at an experimentally determined concentration (0.03 per cent. in water), which gave comparable stimulation responses to those observed in infected plants. It was noted that adventitious root formation occurred in much greater abundance over a somewhat localized zone on the stem close to the point of uptake of the growth substance. The systemic nature of bacterial invasion causing wilt makes this difference quite understandable. Where growth substance was introduced into the vascular system of plants and travelled with the transpiration stream, the systemic invasion effect was paralleled in so far as leaf epinasty and adventitious root formation were concerned. Frequently stem bending was observed in tomato plants treated with growth substance, but no comparable effect has been seen in invaded plants. Swelling of the stem and increased cambial activity, after application of growth substance, has sometimes been paralleled in infected plants. Hunger (1901) recorded the development of adventitious roots on the petioles of plants infected by *B. solanacearum* and roots appear in this position on tomato plants treated with β -indole-acetic acid. Under normal glasshouse conditions the roots induced by the growth substance do not develop beyond the nodular stage, but under humid conditions they break through the epidermis and grow strongly out into the moist air for a distance of 1 to 2 inches. The time for this development, from the date of uptake of growth substance, varied between six and twelve days. When the plants were placed under humid conditions at the stage when nodules were

showing at the surface, two to three days sufficed for strong outgrowth. These times were similar to those for outgrowth of the roots in plants infected by *B. solanacearum*. When growth substance in aqueous solution was allowed to drain into stems of infected plants, which were already showing some degree of leaf epinasty and adventitious root formation, an additive effect was obtained in that more roots grew out and previously unaffected leaves developed epinasty. When growth substance in lanoline was smeared along the lower surface of a bacterially reflexed petiole the epinasty was overcome, and when the concentration was increased such previously reflexed petioles assumed a hyponastic position.

Histological Observations on Bacterially Induced Roots.

Considerable interest attached to determining whether the induction of adventitious roots by *B. solanacearum* was due to local action or to action at a distance. Hutchinson (1913) and Smith (1914, 1920) were of the opinion that the development of adventitious roots occurred in the local absence of bacteria. Both show photomicrographs of developing roots and point out that no bacteria are present in the vessels behind them. From a detailed histological study of numerous infected plants the author believes that the picture presented by these workers must be modified. In transverse sections all conditions were found ranging from heavy bacterial blocking in vessels immediately behind the developing root to complete absence of bacteria. In the majority of cases, however, for tomato, sunflower, and African marigold stems, the bacteria were present in some vessels in the regions where adventitious roots were developing. Frequently the bacteria-filled vessels as viewed in transverse section were separated radially from the zone of root development by three to four locally non-invaded vessels, while in other instances bacteria were present in vessels on either side of the embryonic root, while the vessels immediately behind it were free. Typical conditions are represented in fig. 1 and Pl. XVI., fig. 5.

Transverse sections, however, gave only a limited picture of the relation of the bacteria to the adventitious roots. A much clearer and more complete picture was given by combining observations on transverse sections with the examination of series of longitudinal sections, and observations on the path of vessel invasion as determined using a staining and maceration method (Grieve, 1936b). Using this latter method it was possible to demonstrate with a minimum of labour that the majority of the adventitious roots developed approximately along the lines of the larger primary bundles which were being invaded. In only rare instances did roots develop close to invaded median trace bundles.

Where infected plants were large and a considerable amount of secondary growth had taken place, adventitious roots were found at points other than the large primary bundles. A study of longitudinal and transverse sections through stem regions bearing adventitious roots at various degrees of development (i.e., from root primordia in the incipient stage to well developed nodules visible at the surface) showed the following relations of the

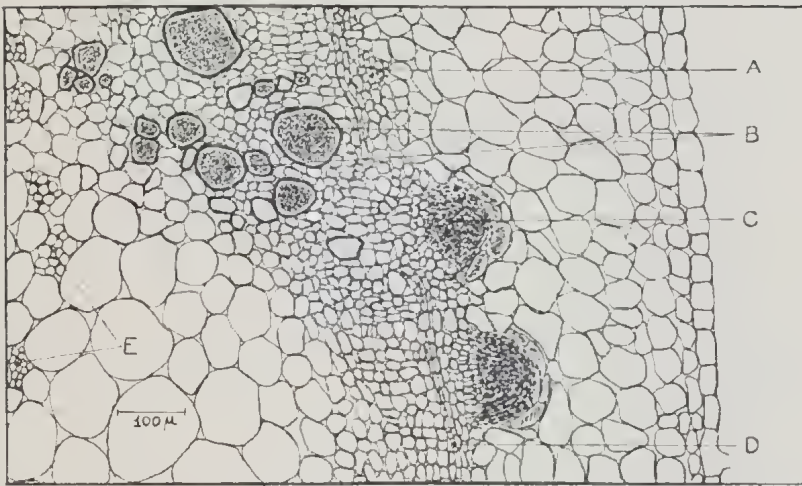


FIG. 1.—Transverse section of tomato stem showing adventitious roots in relation to invaded vessels. A = external phloem, B = vessels full of bacteria, C = Adventitious root, D = cambium, E = internal phloem. Camera lucida drawing.

bacteria to the roots:—(a) Adventitious roots frequently commenced to develop ahead of advancing columns of bacteria in vessels. (b) Bacteria were often found growing in the vessels closest to the stimulated root, but the presence of bacteria even in vessels which were separated by other tissues from the region of root development, does exert a stimulating effect (fig. 2). (c) Development of root primordia once initiated continues to the stage where the root becomes visible as a nodule at the surface of the stem, even though the bacteria during this period gradually block those vessels nearest the incipient root. Bacteria have even been observed passing into the tracheal system of a young root without immediately inhibiting its development (Pl. XVI., fig. 5). (d) Where most of the vessels in a main primary bundle were showing bacterial invasion, root primordia were stimulated to develop on either side of the blocked bundle (fig. 1). (e) Where the speed and completeness of invasion is very rapid so that

vessels are filled with the pathogen within a few days, leading to severe wilting, no adventitious roots have been observed to develop.

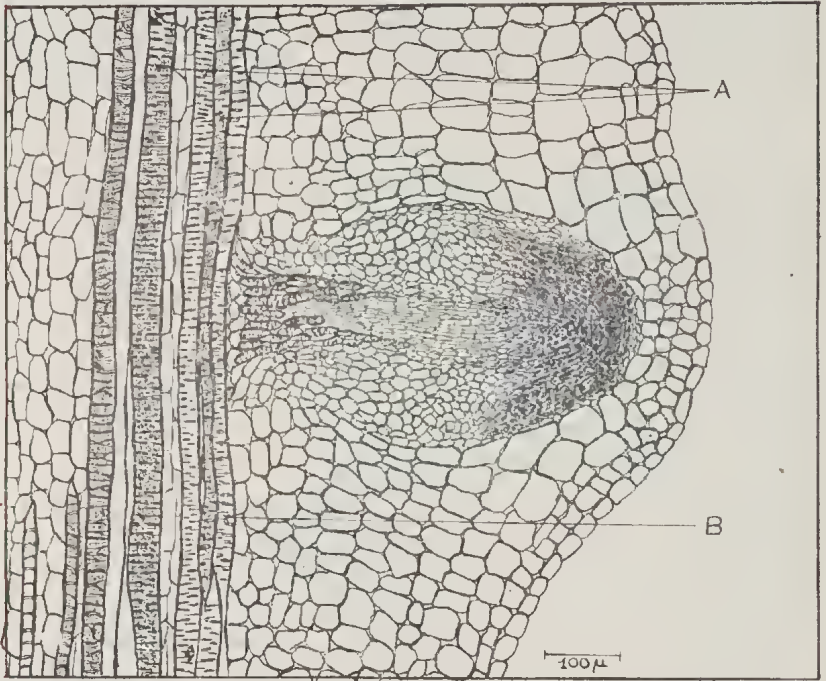


FIG. 2.—Longitudinal section through tomato stem showing relation to the bacteria to the young root. A = vessels in which bacteria are present, B = vessel closest to root primordium. No bacteria are present. Camera lucida drawing.

Growth Substance and Adventitious Root Formation.

The production of adventitious roots, stem swelling and leaf epinasty in plants infected by *B. solanaccarum* parallels these effects induced in healthy plants by the application of growth substances such as β -indole-acetic acid. This raises the question as to whether growth substance is associated with the formation of adventitious roots in such infected plants. In an attempt to answer this, the growth substance content of equivalent stem portions of healthy and infected tomato plants, which were of closely similar size and equal vigour prior to inoculation, was compared. The agar diffusion method method of extraction of growth substance was used in preliminary experiments, but was discarded later in favour of the ether extraction method. Sixteen technically successful experiments were carried out but

only in five were there appreciable differences between the content of growth substance of healthy and infected plants. The results are set out in Table II.

TABLE II.—DIFFERENCE IN PLANT UNITS (AS PERCENTAGE OF CONTROL) BETWEEN THE CONTENT OF GROWTH SUBSTANCE OF HEALTHY AND INFECTED PLANTS.

EXPERIMENT NUMBER.	DIFFERENCE.
3, 7, 18, 20, 22	+ 13, + 14, - 27, - 44, + 50
10, 12, 17, 19, 21	+ 1.5, + 1.4, - 2.1, + 3.5, - 1.4
4, 8, 13, 15, 14	3, - 1.9, 3, 2, 0

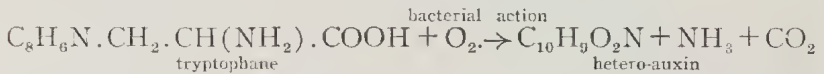
+ Indicates that infected plants contain more than the control. The total content of the controls varied from 67 to 198 plant units.

In spite of the large positive differences recorded in the first group of experiments, statistical examination shows that the difference between infected and healthy plants is not significant (either at the 1 or the 5 per cent. level). Further experiments of this kind are in progress, but it should be borne in mind that very small amounts of active substance suffice to cause marked stimulation effects and also that some of it would be used up in initiating the root primordia.

Should it eventually be found that there is a significantly greater amount of growth substance in infected plants showing root formation, it would still be necessary to discover its origin, that is, to find out whether it was produced as a result of bacterial metabolism or by the host cells as a reaction to invasion. The approach to this problem must necessarily be indirect. In the case of Crown Gall some workers have shown that *B. tumefaciens* could produce growth substance in culture media. Brown and Gardner (1936-37) and Link, Wilcox and Link (1937) inclined to the view that part at least of the stimulation effect was due to the production of heteroauxin by the pathogen. It had been shown earlier (Grieve, 1939), that *B. solanacearum* produced a growth substance in culture media containing peptone, glucose and mineral salts. Further studies showed that both virulent and non-virulent cultures of *B. solanacearum* produced approximately equal amounts of growth substance as determined by the *Avena* test. The same result was obtained when pathogenic and non-pathogenic cultures of *Aplanobacter michiganense*, *B. tumefaciens*, and *B. flaccumfaciens* were tested. The active substance, extracted in crude form, gave positive tests for heteroauxin and induced adventitious roots on application to tomato and African marigold plants (Pl. XVI., fig. 3). The fact that all the organisms named above produce growth substance in culture

media irrespective of whether they are pathogenic or non-pathogenic, or of whether they produce stimulation effects in their host plants, makes it doubtful, that such a mechanism is necessarily involved in infected plants. Moreover, as Locke, Riker, and Duggar (1938) observed in the case of Crown Gall, the volume of culture which must be extracted and the number of bacteria present to give a small amount of growth substance, greatly exceeds the volume of sap and the number of bacteria present in the invaded plants, where the stimulatory effect is more marked.

On the question of the possible production of heteroauxin by the bacteria in the invaded vessels, more direct evidence was sought by an experiment which involved the uptake of the amino acid tryptophane into plants. The rationale for this experiment was as follows:—The production of growth substance (heteroauxin) by bacteria in media containing tryptophane follows the course of oxidative de-amination (Thimann, 1935).



If such a process of oxidative de-amination was taking place in the invaded vessels where the amino acid tryptophane might be expected to be present, it seemed reasonable to assume that an artificial increase in the concentration of this substance would lead to a greater production of growth substance by the bacteria and the presence of this in turn would be reflected by more pronounced epinasty of leaves and adventitious root formation. To test this the following procedure was adopted. Ten tomato plants of closely similar size and of equal vigour were all inoculated with standard inoculum at the same height in a main bundle. Two days later 0.4 cc. of a 0.5 per cent. concentration of l-tryptophane was allowed to drain from small tubes into the inoculated bundles of five of the test plants. At the same time 0.4 cc. of water was allowed to drain into the inoculated bundles of the other five plants. Plants were examined from day to day for epinasty of leaves and adventitious root formation. No evidence was obtained, however, to indicate that any increased production of growth substance due to the addition of tryptophane occurred, the degree of epinasty and of adventitious root formation being approximately the same in both series. Wilting supervened earlier in the plants supplied with tryptophane and sections showed more bacteria in the vessels of these plants than in those of the control plants. Since 0.4 cc. of a 0.5 per cent. concentration of tryptophane when allowed to drain into a healthy plant has no deleterious effect, it is concluded that the tryptophane in the above experiment was utilized as a food source by the parasite and the increased numbers of bacteria caused the earlier wilting of the plants. This experiment lends no support to the hypothesis

that *B. solanaccarum* produces growth substance by acting on naturally occurring tryptophane in the xylem sap. It is possible that under certain conditions tryptophane present in the vessels may be acted upon to give heteroauxin, but the above result, taken in conjunction with the results for growth substance formation in culture media, makes it appear unlikely that the production of heteroauxin by the parasite plays any major role in the induction of adventitious roots in infected plants.

The alternative view is that the stimulation effects might be due to excessive production of growth substance by the plant tissues under the influence of the bacteria (suggested in the case of Crown Gall by Leonian (1937), and Locke, Riker and Duggar (1938)), or to its local accumulation in the tissues. Data bearing on these possibilities have been obtained from experiments involving artificial blocking of vessels. Details of these will first be considered before passing to the hormone mechanism. It has been shown earlier in this paper that bacteria are present, generally in blocking numbers, in some stem vessels of tomato, below the points where adventitious roots commence to develop and it appeared desirable to test whether mechanical blocking could induce root formation.

The conditions of bacterial blocking were found difficult to duplicate as any attempt to block the xylem vessels involved interruption of at least the external phloem. This difficulty was partly obviated by combining experiments involving interruption of xylem and phloem by cutting, with others in which blocking substances were introduced into the xylem after cutting. The cut stems served in the sense of controls to the cut and blocked stems. A positive result was obtained, as in several experiments roots developed for over 1 inch both above and below cut bundles blocked with such substances as cocoa butter, lanoline and paraffin wax-vaseline mixture, while in the control plants where only cutting was practised, adventitious roots were found to develop only in the immediate vicinity of the cut. In experiments reported earlier on mechanical blocking in relation to epinasty (Grieve, 1939) positive results were obtained in only three out of 40 experiments. It is of interest to note that further examples of epinasty induction by blocking were recorded in the present experiments, but the condition was not found to be constantly associated with the experimentally induced adventitious roots.

The results obtained in these blocking experiments point to the possibility that where the invading bacteria are present in large numbers sufficient to block some of the vessels, the blocking effect may be an important factor in root formation. However, until a method of blocking the vessels with inactive substances without interfering with the phloem is developed, judgment must be reserved as to the closeness of the parallel and the importance of mechanical blocking.

The formation of adventitious roots above a cut on a stem has been explained on a plant hormone basis, as being due to the prevention of longitudinal transport of growth substance or root substance (Boysen-Jensen, 1936) and this explanation would appear to apply also in the case of the cut stems of tomato. In the cut and blocked bundles, the additive effect recorded may be explained either by envisaging a heightened production of growth substance in the vicinity of the affected parts or an accumulation of growth substance coming from other parts via either xylem or phloem. The first hypothesis appears to fit better the fact that adventitious roots form in the blocked region below a cut.

While as indicated above, caution must be exercised in making too close a parallel between the cutting and blocking of bundles of stems and the bacterial blocking of xylem, yet the results obtained, taken with the negative results for heteroauxin formation in the vessels, make it probable that the stimulation effects are due to an increase in local concentration of growth substance produced by the host cells which concentration is brought about by the presence of the parasite.

Disturbance of xylem-phloem relations by blocking of vessels and the accumulation in them of metabolic products (which are non-active physiologically) may both contribute to the increase in growth substance content leading to the formation of adventitious roots.

Summary.

1. A detailed account has been given of the development of adventitious roots in plants infected by *Bacterium solanacearum*. Comparisons have been made with similar effects induced by *Bacterium tumefaciens* and *Aplanobacter michiganense*, by synthetic growth substance (heteroauxin), by wounding and blocking of stems and by the influence of gravity. The relation of the invading bacteria to the developing roots was established by transverse and longitudinal sections, showing that the root primordia commenced development ahead of the advancing columns of bacteria in the vessels. Development continued to the nodule stage even though the bacteria filled most of the vessel behind the root primordium.

2. Results of ether extractions gave no significant difference in the growth substance content of healthy and invaded stem parts. Nevertheless the close parallel between the formation of adventitious roots in infected plants and by synthetic growth substance, indicated that growth substance was associated with the adventitious root primordia. The question as to whether the active substance inducing the roots is produced by the pathogen or the host cells is discussed. The evidence so far available indicates that it is more likely that the stimulation effects are due to the increase or accumulation of plant hormone under the influence of the invading bacteria, rather than to a direct product of bacterial metabolism.



1



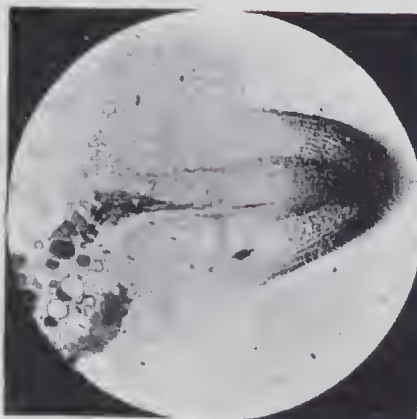
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5

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Explanation of Plate.

PLATE XVI.

- FIG. 1.—Infected tomato plant showing outgrowth of adventitious roots in humid air.
- FIG. 2.—Adventitious roots induced in *Tagetes erecta* on infection by *B. solanacearum*.
- FIG. 3.—Production of adventitious roots in *Tagetes erecta* after uptake of growth substance extracted from media in which *B. solanacearum* had grown.
- FIG. 4.—Tomato stems infected by *B. solanacearum* showing outgrowth of roots when the cut bases are placed in water.
- FIG. 5.—Transverse section of a developing adventitious root on an infected tomato stem. Note the presence of bacteria in the vessels leading to the vascular system of the new root.